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Plasmid Mid Kit Liquid Form

Product Number: PLK0801

Shipping and Storage

1. When using for the first time, add all RNase A carried by the reagent kit to Buffer P1 (concentration 100μg/ml) and store at 4℃. If RNase A is inactivated in Buffer P1, the extracted plasmid may contain trace amounts of RNA residue. Adding RNase A to Buffer P1 is sufficient.
2. Buffer ER can be stored at 4℃ for one month. If it needs to be stored for a long time, it is recommended to store it at -20℃! When the ambient temperature is low, SDS in Buffer P2 may precipitate and become turbid or precipitate. It can be heated in a 37℃ water bath for a few minutes to restore clarity. Do not shake violently to avoid excessive foam formation.
3. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	PLK0801 20 Preps	PLK0802 40 Preps
RNase A(10mg/ml)	-20℃	0.75 ml	1.3 ml
Buffer P1	4℃	65 ml	130 ml
Buffer P2	RT	50 ml	100 ml
Buffer P3	RT	50 ml	110 ml
Buffer IRA	RT	1.5 ml	3 ml
Buffer IRB	RT	15 ml	30 ml
Buffer ER	-20℃	5 ml	10 ml

Description

This reagent kit extracts plasmid DNA from cultured bacteria using alkaline lysis method, using a unique solution formula and Buffer ER. It only requires a few simple centrifugations to remove impurities such as proteins, polysaccharides, endotoxins, RNA, etc., to obtain high-quality plasmid DNA. The OD260/280 of purified DNA is usually around 1.8, and the resulting plasmid can be directly applied in tasks that require high DNA purity, such as cell transfection and even animal in vivo experiments. The purification process in the later stage is operated in a 1.5ml small centrifuge tube, which is simple, does not require special equipment, does not require column passing, and does not require phenol chloroform extraction; Plasmids released by bacterial lysis can be completely recovered without worrying about the loss of plasmid DNA. This method extracts and purifies plasmid DNA with minimal damage to plasmids. Even large plasmids or ultra large BAC/PAC plasmids with a size of 10kb or even 100kb can be effectively purified as long as they can be extracted by alkaline lysis. You can choose to dissolve plasmids in any small volume, with a concentration of up to 5μg/μl. The super helix ratio can reach up to 95%, with no endotoxins and good transfection effect.

Features

1. No toxic reagents such as phenol and chloroform are needed, and no ethanol precipitation is required. Fast and convenient extraction of 150μg-600μg pure high copy plasmid DNA from 50-70ml of Escherichia coli LB (Luria Bertani) culture medium, with an extraction rate of 80-90%.
2. The obtained plasmids have high yield, concentration, super helix ratio, and purity, and can be directly used for various molecular biology experiments such as enzyme digestion, transformation, PCR, in vitro transcription, sequencing, etc.
3. The endotoxin content is extremely low (<0.1EU/μg DNA) and can be directly applied to cell transfection.

Application

Suitable for medium to high purity or transfection level plasmid preparation and BAC/PAC large-scale plasmid preparation

Note

1. The amount of plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number. If the extracted plasmid is a low copy plasmid or a large plasmid larger than 10kb, the amount of bacterial cells used should be increased, and the amount of Buffer P1, P2, and P3 should be increased proportionally.
2. When extracting large plasmids, the operation should be gentle and a suction head with an enlarged opening should be used to prevent mechanical cutting from damaging DNA.
3. After centrifugation of DNA precipitation solution, obvious precipitation may not be visible. If no precipitation is observed and there is concern about DNA loss, the supernatant can be retained. After completing all operations, electrophoresis identification can be performed to determine whether the final product has been obtained (several μg of DNA centrifuged and precipitated on the sidewall of the tube, and obvious clumps may not be visible).
4. The concentration and purity of the obtained plasmid DNA can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. A OD260 value of 1 is equivalent to approximately 50μg/ml of DNA. Electrophoresis may consist of a single band, as well as 2 or more DNA bands, mainly due to varying degrees of superhelix conformational plasmids swimming in different positions, which are related to the length of extract culture time and the intensity of extraction operations. Under normal operating conditions, our company's products can basically exceed 95% over rotation.
5. The exact molecular size of plasmid DNA can only be determined by comparing DNA molecular weight markers after enzyme tangent normalization. Plasmids in a circular or super spiral state have an uncertain swimming position and cannot be determined by electrophoresis for exact size.

Protocol(Please read the precautions before the experiment)

Tip: Add all RNase A to Buffer P1, mix well, and store at 2-8℃ after use.

1. Take 40-60ml (maximum not exceeding 90 ml) of overnight cultured bacterial solution and transfer it into a 50ml centrifuge tube. Centrifuge 4500-6000×g at 4℃ for 5 minutes to precipitate the bacterial body (or 12000×g for 2 minutes), and completely discard the supernatant.
2. Add 2.5ml Buffer P1, thoroughly suspend and shake the bacterial precipitate to completely disperse, and let it stand at room temperature for 3-5 minutes until there are no flocs.

   If there are incompletely mixed bacterial blocks, it will affect the lysis, resulting in low extraction amount and purity.
3. Add 2.5ml of Buffer P2, gently invert the centrifuge tube 6-8 times, and let it stand at room temperature for 5 minutes until the bacteria completely lyse and the solution becomes transparent.

   Mix gently and do not shake vigorously to avoid genomic DNA cleavage! The time used should not exceed 5 minutes! To prevent plasmid damage. At this point, the bacterial solution should become clear and viscous. If there are few bacterial cells, they can quickly become clear and viscous before proceeding to the next step, not necessarily accurate for 5 minutes. If it does not become clear, it may be due to excessive bacterial growth and incomplete lysis, and the bacterial population should be reduced.
4. Add 2.5ml of Buffer P3, immediately invert the centrifuge tube 6-8 times, mix well until white flocculent material is produced. The above lysis solution was centrifuged at 4℃ for 10-15 minutes at 12000-16000×g, and the supernatant was carefully aspirated and transferred into a new 50ml centrifuge tube.

   After adding Buffer P3, it should be mixed immediately to avoid local precipitation of SDS,

   Note: When using isopropanol precipitation, there is less co precipitation of protein impurities and salt ions, and there may not be obvious large clump precipitation. However, the plasmid can still be completely precipitated, which does not affect the actual plasmid yield. If you are accustomed to seeing larger precipitate clumps, you can choose to precipitate with 2 times the volume of anhydrous ethanol.
5. Add 5ml of isopropanol, invert the centrifuge tube, and mix thoroughly.
6. Centrifuge at 4 ℃ 12000-16000×g for 10 minutes, carefully discard the supernatant, pour it upside down on absorbent paper,

gently drain the residual liquid, add 3-5 ml of 70% ethanol to rinse once, centrifuge at the highest speed for 5 minutes, discard the supernatant, and air dry the precipitate.

If DNA precipitation is too dry, DNA will not be completely dissolved. However, if ethanol is not dried and evaporated completely, too much residue will also cause DNA to not be completely dissolved.

Note: After centrifugation and precipitation with isopropanol, the purity of the plasmid is very high. It may not be visible on the bottom and side walls of the tube, but it does not affect the yield. In the subsequent steps, carefully blow the bottom of the tube and rinse the side walls where the precipitate is located to dissolve the plasmid.

7. Add 0.7ml Buffer P1 to completely dissolve the precipitate clump. Note that although the plasmid precipitate attached to the bottom and side walls of the tube may not be visible, it should be rinsed off by blowing the bottom of the tube and the side wall where the precipitate is located (large plasmids can be dissolved by gently blowing with a wide mouthed straw). Then transfer the plasmid solution into a new 1.5ml centrifuge tube.

   Optional steps (generally not required): If the strain has abundant RNA and trace RNA residues, the plasmid solution can be incubated at 60 ℃ for 15 minutes after this step to digest the RNA.

8. Add 55μl Buffer IRA, invert and mix thoroughly, then add about 0.1 volume (about 80μl) of ice pre cooled Buffer ER, invert and rotate 7-10 times (about 30 seconds) to mix thoroughly. Place in an ice bath or on ice for ≥ 5 minutes, occasionally invert and mix a few times in between.

   After adding buffer ER to the supernatant, it will become turbid, but it should return to a clear state after ice bath.

   Note: If it is not necessary to remove endotoxins for transfection, only 55μl Buffer IRA can be added in this step. Mix well and leave on ice for 5 minutes. After centrifugation, carefully transfer the supernatant into a new tube and proceed directly to step 11.

9. 42 ℃ water bath, the solution will become turbid again, mix upside down and incubate at 42℃ for 5 minutes.

10. Centrifuge at room temperature of 14000×g for 5 minutes to separate phases (when the temperature is low, the Buffer ER cannot separate phases, so it must be centrifuged at room temperature of at least 20℃ or ensure that the winter rotation temperature is above 20℃). The upper aqueous phase contains DNA, while the lower blue oily phase contains endotoxins and other impurities. Transfer the upper aqueous phase containing DNA to a new tube (be careful not to inhale the blue oily layer, which contains impurities such as endotoxins), and discard the oily layer.

    The solution must be divided into upper and lower phases, otherwise steps 9-10 should be repeated.

11. Add an equal volume of Buffer IRB (about 750μl) to the upper aqueous phase obtained in the previous step, mix gently, centrifuge at 4℃ for 10 minutes at 14000×g, discard the supernatant (be careful not to lose DNA), gently add 1ml of 70% ethanol for washing, centrifuge and discard the supernatant twice, and air dry at room temperature for 5-10 minutes until the ethanol completely evaporates.

12. Add an appropriate amount of TE or pure water (50-100μl) to dissolve the precipitate (it can be shaken in a 37 ℃ water bath to assist dissolution).

    It should be noted that many plasmid DNA may adhere to the sidewall of the centrifuge tube. Even if it is not visible, the sidewall should be thoroughly blown to dissolve and recover the plasmid DNA

    The final precipitation can be dissolved in any small volume as needed, resulting in a high concentration of transfected plasmid DNA (up to 5-10μg/m). If necessary, customers can also choose a larger volume for dissolution.